INTRODUCTION
Most therapeutic models for spinal cord injury are aimed at neural regeneration and ignore the underlying inflammatory process associated with spinal cord injury. Inflammation is normally a protective attempt to facilitate the removal of damaged tissue and cellular debris and to initiate the healing process. However, in the spinal cord this response is perturbed, leading to chronic inflammation, secondary degenerative processes and glial scarring. We propose that the stimulation of pro-inflammatory pathways to enhance early phagocytic activity, and subsequently alter inflammatory cell phenotypes from pro- to anti-inflammatory, might be a strategy to block the adverse effects of chronic inflammation seen in spinal cord injuries. Early pro-inflammatory signals may be necessary as insufficient phagocytosis will result in a continued disruption of the blood-brain barrier and prolonged inflammation, leading to a non-permissive environment for regeneration. A threshold must be reached whereby the level of monocyte infiltration and activation is able to cope with the level of cell apoptosis and ultimately tip the balance in favour of inflammatory resolution. Completing the inflammatory cycle and enabling the timely resolution of inflammation may be an important part of regeneration strategies in the central nervous system. To this end we have treated acute spinal cord injuries with vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF) delivered from a hydrogel patch to modulate early inflammatory events.

METHODS

Growth Factor Patch Preparation and Application Recombinant human PDGF-BB (5µg) and recombinant mouse VEGF-165 (5µg) were encapsulated in a Flucon P127/PEG800/PEGdiacrylate interpenetrating network gel, which was photopolymerized at room temperature. Release of each growth factor was quantified using commercially available ELISA kits. A gel control patch was prepared that contained no growth factors

Animal Surgery- Lesion Appropriate animal ethics approvals was obtained from the Queensland University of Technology and the Griffith University animal ethics committees. Adult male Wistar rats (400g; 20-25 weeks old) were anaesthetised with intraperitoneal injection of 70 mg/Kg Zoletil 100 and 20mg/Kg Xylazine. Complete laminectomy were performed at T10. The animals subjected to T10 hemisections using a 29G needle sectioning from the midline out. Animals were randomised to a treatment (active gel) or control groups (lesion control or gel control) after the spinal cord injury had been induced. Animals were sacrificed 1 and 3 months post lesion.

Immunofluorescence Histology Sections were washed with PBS-Triton (0.1% Triton X 100 (Merck, Germany) in PBS) in 10 min, permeabilised in dimethyl sulfoxide for 20 min and washed again. Sections were blocked with 10% donkey serum (Sigma) and stained with antibodies to Neurofilament-200, Glial Fibrillary Acidic Protein, microglia/macrophage with IBA-1. Sections were incubated with the Alexa Fluor 594 labelled Donkey anti-rabbit secondary antibodies (Molecular Probes) overnight at room temperature and mounted with Vectorshield mounting media with DAPI (Vector Laboratories). Mounted sections were analysed on a Zeiss Z1 microscope with ApoTome with a digital camera attached to a computer.

RESULTS
The release of the growth factors from the patch was complete by 24 hours. When examined both 30 and 90 day later the Active Gel-treated animals had a significantly smaller lesion cavity (Fig 1 and Fig 2). The mean lesion size for the lesion control group was 2.09mm², 1.97mm² for the gel control group and 0.45mm² for the active gel group. An ANOVA was used to confirm that the differences between the active gel and the two control groups were statistically significant (AG vs LC p= 0.021 AG vs GC p= 0.026).

DISCUSSION
We observed a marked reduction in the glial scar after Active Gel treatment, indicated by the reduction in astroglisis and increased neurofilament immunoreactivity, strongly suggesting a resolution of chronic inflammation. There was a clear difference in the morphology of the axons and astroglia up to 200µm distal to the lesion site. In the Active Gel-treated cord astrocytes are more sparse with a large number of processes. In the Lesion Control the numbers of astrocytes are increased and most are stunted with absent processes and spindle shaped cell bodies, typical of reactive astrocytes found in both acute and chronic lesions. In the Active Gel-treated cord there is more neurofilament immunoreactivity (NF200) typical of healthy axons, compared to the sparse, punctate staining in the Lesion Control. The reduction in lesion size and number and size of cystic cavities is evident one month after Active Gel treatment compared to controls. We hypothesise the delivery of VEGF significantly increases the permeability of the blood spinal cord barrier to neutrophils and monocytes and the codelivery of PDGF stimulates chemotaxis, proliferation, and new gene expression in monocytes-macrophages, thereby increasing the initial influx of inflammatory cells. These growth factors appear to moderate the secondary degenerative changes that results from the prolonged inflammation inherent to the injured cord and promote regeneration.

Reduction of Secondary Degeneration after Spinal Cord Injury by Acute Delivery of Vascular Growth Factors
Cameron Lutton 1, Yun Wai Young 2, Adrian C. B. Meedeniya 2, Alan Mackay -Sim 2, +Ben Goss 3
1Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia  2National Adult Stem Cell Centre, Griffith University, Brisbane, Australia  3AOspine Reference Centre, Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia
Senior author b.goss@qut.edu.au

Figure 1: Mean lesion size measured from histological sections of hemisected spinal cord 3 months post lesion randomized to a treatment group (n=5

Figure 2: Spinal cords harvested at 3 months post lesion randomized to (a) Lesion Control (b) Active Gel (gel control not shown). All sections were stained with NF200 (red) GFAP (green) and DAPI (blue). The lesion site is inside the box in (b)